

**Amendments to the Specification:**

At page 1, please replace the Title with the following amended Title:

**~~An Extract of a Plant and Its Cosmetic Use~~ COSMETIC COMPOSITIONS  
CONTAINING AN EXTRACT OF LEAVES OF THE CASTANEA SATIVA PLANT AND  
COSMETIC TREATMENTS .**

At page 1, in the line after the Title, enter the following new heading and paragraph:

**RELATED APPLICATIONS**

This application is filed under 35 U.S.C. § 371 claiming priority from application PCT/EP2005/001105 filed February 4, 2005, which claims priority from European application EP 04290388.0 filed February 13, 2004; the entire contents of each document are incorporated herein by reference.

Please add the following heading on page 1, before line 1:

**FIELD OF THE INVENTION .**

Please add the following heading on page 1, before line 6:

**BACKGROUND OF THE INVENTION .**

Please add the following heading on page 3, before line 17:

**BRIEF DESCRIPTION OF THE INVENTION .**

Please add the following new heading on page 3, before line 24:

**DETAILED DESCRIPTION OF THE INVENTION.**

Please replace the paragraph beginning on page 6, line 6, with the following amended paragraph:

Surfactants (or Surface-active substances) that may be present are anionic, non-ionic, cationic and/or amphoteric ~~or amphoteric~~ surfactants, the content of which in the compositions is usually about 1 to 70% by weight, preferably 5 to 50% by weight and in

particular 10 to 30% by weight. Typical examples of anionic surfactants are soaps, alkylbenzenesulfonates, alkanesulfonates, olefin sulfonates, alkyl ether sulfonates, glycerol ether sulfonates,  $\alpha$ -methyl ester sulfonates, sulfo fatty acids, alkyl sulphates, fatty alcohol ether sulphates, glycerol ether sulphates, fatty acid ether sulphates, hydroxy mixed ether sulphates, monoglyceride (ether) sulphates, fatty acid amide (ether) sulphates, mono- and dialkyl sulfosuccinates, mono- and dialkyl sulfosuccinamates, sulfotriglycerides, amide soaps, ether carboxylic acids and salts thereof, fatty acid isethionates, fatty acid sarcosinates, fatty acid taurides, N-acylamino acids, e. g. acyl lactylates, acyl tartrates, acyl glutamates and acyl aspartates, alkyl oligoglucoside sulphates, protein fatty acid condensates (in particular wheat-based vegetable products) and alkyl (ether) phosphates. If the anionic surfactants contain polyglycol ether chains, these may have a conventional homologous distribution, but preferably have a narrowed homologous distribution. Typical examples of non-ionic surfactants are fatty alcohol polyglycol ethers, alkylphenol polyglycol ethers, fatty acid polyglycol esters, fatty acid amide polyglycol ethers, fatty amine polyglycol ethers, alkoxyated triglycerides, mixed ethers or mixed formals, optionally partially oxidized alk(en)yl oligoglycosides or glucuronic acid derivatives, fatty acid N-alkylglucamides, protein hydrolysates (in particular wheat-based vegetable products), polyol fatty acid esters, sugar esters, sorbitan esters, polysorbates and amine oxides. If the non-ionic surfactants contain polyglycol ether chains, these may have a conventional homologous distribution, but preferably have a narrowed homologous distribution. Typical examples of cationic surfactants are quaternary ammonium compounds, e. g. dimethyldistearyl-ammonium chloride, and ester quats, in particular quaternized fatty acid trialkanolamine ester salts. Typical examples of amphoteric or zwitterionic surfactants are alkylbetaines, alkylamidobetaines, aminopropionates, aminoglycinates, imidazolinium-betaines and sulfobetaines. Said surfactants are known compounds. With regard to structure and preparation of these substances, reference may be made to relevant review works.

Please replace the paragraph beginning on page 11, line 6, with the following amended paragraph:

Fats and waxes that can be used are described in the following text. Typical examples of fats are glycerides, i.e. solid or liquid vegetable or animal products which consist essentially of mixed glycerol esters of higher fatty acids, suitable waxes are inter alia natural waxes, for example candelilla wax, carnauba wax, japan wax, esparto grass wax, cork wax, guaruma wax, rice germ oil wax, sugarcane wax, ouricury wax, montan wax, beeswax, shellac wax, spermaceti, lanolin (wool wax), uropygial grease, ceresin, ozokerite (earth wax), petrolatum, paraffin waxes, microcrystalline waxes; chemically modified waxes (hard waxes), for example montan ester waxes, sasol waxes, hydrogenated jojoba waxes, and synthetic waxes, for example polyalkylene waxes and polyethylene glycol waxes. In addition to the fats, suitable additives are also fat-like substances, such as lecithins and phospholipids. The term lecithins is understood by the person skilled in the art as meaning those glycerophospholipids which form from fatty acids, glycerol, phosphoric acid and choline by esterification. Lecithins are thus frequently also ~~{lacuna}~~ known as phosphatidylcholines (PC). Examples of natural lecithins which may be mentioned are the cephalins, which are also referred to as phosphatidic acids and represent derivatives of 1,2-diacyl-sn-glycerol-3-phosphoric acids. By contrast, phospholipids are usually understood as meaning mono- and, preferably, diesters of phosphoric acid with glycerol (glycerophosphates), which are generally considered to be fats. In addition, sphingosines and sphingolipids are also suitable.

Please replace the heading on page 18, line 19, with the following amended heading:

~~**Process for the aqueous extraction of Castanea sativa leaves**~~ **PROCESS FOR THE  
AQUEOUS EXTRACTION OF CASTANEA SATIVA LEAVES**

Please replace the paragraph on page 18, beginning on line 21, with the following amended paragraph:

0.2 kg of dried and ~~powered~~ powdered Castanea sativa leaves were introduced into a beaker containing 2 l of distilled water. The mixture was then stirred at 60 °C for an hour. The solids were removed by centrifugation (4200 rpm for 15 minutes) and filtration. The filtered solution formed a crude solution of the extract, the water was removed by spray drying and the obtained yield was 6.22 % of the weight of the dried leaves. The extract thus obtained is called "aqueous extract". It was used for the experiments which are described

in the following text. The extraction was carried out twice resulting in two aqueous extracts: "batch A" and "batch B".

Please replace the heading on page 18, line 30, with the following amended heading:

~~Constituents found in this aqueous extract~~      **CONSTITUENTS FOUND IN THIS AQUEOUS EXTRACT**

Please replace the heading on page 18, line 35, with the following amended heading:

~~Process for the alcoholic extraction of Castanea sativa leaves (80 volume-% methanol + 20 volume-% water)~~      **PROCESS FOR THE ALCOHOLIC EXTRACTION OF CASTANEA SATIVA LEAVES (80 VOLUME-% METHANOL + 20 VOLUME-% WATER)**

Please replace the heading on page 19, line 10, with the following amended heading: ~~Example 1: Anti-free radicals activities (this experiment is analogous to an experiment described in WO 02/080949)~~      **EXAMPLE 1: ANTI-FREE RADICAL ACTIVITY (THIS EXPERIMENT IS ANALOGOUS TO AN EXPERIMENT DESCRIBED IN WO 02/080949)**

Please replace the paragraph beginning on page 19, line 24, with the following amended paragraph:

**1.2 Methods and principle of this experiment:** The anti-free radical (anti-FR) activity has been evaluated by chemical tests for the evaluation of the potential to scavenge free radicals  $R^\bullet$  (~~DPPH° test~~) R (DPPH test) and hydroxyl radicals  $HO[[^\bullet]]$ . The anti-FR activity has been evaluated also by biochemical tests to address the potential for scavenging of a kind of reactive oxygen species (ROS), the so called super oxide anion ( $[[O_2^\bullet]] O_2$ ). The  $[[O_2^\bullet]] O_2$  originates mainly from xanthine oxydase and lipoxxygenase activities. Xanthine oxydase (XOD) is an enzyme activated during oxidative stress, this enzyme catalyses the  $[[O_2^\bullet]] O_2$  release along the degradation of hypoxanthin (HX) which has been overproduced by a disruption of energetic cell metabolism. Then  $[[O_2^\bullet]] O_2$  dismutates either spontaneously or by superoxyde dismutase (SOD) activity, into hydrogen peroxide ( $H_2O_2$   $H_2O_2$ ), which constitutes a source of  $HO[[^\bullet]]$  following the Fenton's reaction lipoxxygenase activity, displayed by leukocytes, produces also  $[[O_2^\bullet]] O_2$  along the

leukotriens synthesis from arachidonic acid released during inflammatory process (Mac Cord M, Chabot Fletcher M, Breton J, Marshall La, Human keratinocytes possess an sn-2 acylhydrolase that is biochemically similar to the U937-derived 85-kDa phospholipase A2, Journal of Investigative Dermatology, 1994, n° 102, pages 980 to 986 and Bouclier M, Hensby CN, Prostaglandines et leucotriènes en physiologie cutanée, Bulletin d'esthétique Dermatologique et de Cosmétologie, 1986, pages 17 to 22).

Please replace the paragraph beginning on page 20, line 9, with the following amended paragraph:

**Principle:** DPPH<sup>•</sup> (diphenylpicryl-hydrazyl) is a quite stable free radical which forms a purple solution. The purple solution of DPPH<sup>•</sup> has a whitening effect due to components that act as radical-scavengers. The reaction is evaluated by recording the optical density (OD) at 513 nm (DEBY C : C : Relation entre les acides gras essentiels et le taux des antioxydants tissulaires chez la souris : SOCIETE BELGE DE BIOLOGIE, séance du 19 décembre 1970 (year of publication), pages 2675 to 2680).

Please replace the paragraph beginning on page 20, line 24, with the following amended paragraph:

The results are expressed in percent inhibition of DPPH<sup>•</sup> radical against control. The aim of this test is to show an anti-free radical activity.

Please replace the paragraph beginning on page 21, line 4, with the following amended paragraph:

**Principle:** The hydroxyl radical HO<sup>•</sup> (formed by H<sub>2</sub>O<sub>2</sub> H<sub>2</sub>O<sub>2</sub> in presence of Fe<sup>++</sup> with EDTA) oxidises deoxyribose (a component of DNA), then a pink compound is formed by condensation of thiobarbituric acid with oxidised form of deoxyribose. The optical density at 532 nm corresponds to the level of oxidised deoxyribose. An anti-free radical substance reacts with these radicals HO<sup>•</sup> and reduces the formation of this pink compound. This reaction is also carried out without EDTA in order to determine the capacity of ingredient to form an inactive complex with a ferrous ion (Halliwell B, Gutteridge JMC, Aruoma OI, The deoxyribose method: a simple "Test-Tube" assay for determination of rate constants for reactions of hydroxyl radicals, Analytical Biochemistry, 1987, n° 165, p 215-219).

Please replace the paragraph beginning on page 22, line 12, with the following amended paragraph:

Xanthine oxydase is an enzyme induced during oxidative stress: it catabolises the puric bases (adenine, guanine) in uric acid and superoxide anions  $[[O_2-\bullet]]$   $O_2-\bullet$  that dismutates spontaneously (or by the action of SOD = superoxyde dismutase) in  $H_2O_2$   $H_2O_2$  and  $[[O_2]]$   $O_2$ .

Please replace the paragraph beginning on page 22, line 16, with the following amended paragraph:

The superoxide anion  $[[O_2-\bullet]]$   $O_2-\bullet$  can be revealed by NBT (NBT = Nitroblue tetrazolium) by recording the optical density at 540 nm (OHKUMA N et al. : Superoxyde dismutase in epidermis. J. of Invest. Dermatol., 1987, n[°]14, pages 218 to 223) . A substance with an anti-free-radical-activity absorbs or destroys the  $[[O_2-\bullet]]$   $O_2-\bullet$  anion and thereby it reduces the optical density.

Please replace the paragraph beginning on page 23, line 4, with the following amended paragraph:

The results are expressed in percent of inhibition of  $[[O_2-\bullet]]$   $O_2-\bullet$  radical against control. The aim of this test is to show an anti-free radical activity.

Please replace the paragraph beginning on page 23, line 7, with the following amended paragraph:

As to the meaning of U/ml: for an enzyme, the dose is expressed in U/ml Units/ml for unity. In the other cases, it is not enzymes.

Please replace the heading on page 24, beginning at line 19, with the following amended heading:

~~Example 2: Inhibition of UVA effect on human fibroblasts (this experiment is analogous to an experiment described in the European patent application no. 03292802.0)~~ **EXAMPLE 2: INHIBITION OF UVA EFFECT ON HUMAN FIBROBLASTS (THIS EXPERIMENT IS ANALOGOUS TO AN EXPERIMENT DESCRIBED IN THE EUROPEAN PATENT APPLICATION NO. 03292802.0)**

Please replace the paragraph beginning on page 25, line 3, with the following amended paragraph:

**Protocol of the experiment:**

- Seeding of human fibroblasts in a growth medium
- Incubation for 3 days at 37 °C, CO<sub>2</sub> CO<sub>2</sub> = 5 % (atmosphere: 5 % carbon dioxide in air)
- Exchange of the growth medium by a medium with a range of concentration of ingredients to be tested
- Incubation for 2 days at 37 °C, CO<sub>2</sub> CO<sub>2</sub> = 5 %
- Exchange of the medium with ingredients by a balanced salt solution and UV-A irradiation (20 J/cm<sup>2</sup>)
- Recording of released MDA levels by spectro-fluorimetry. (MDA (malonaldehyde) is a product of oxidative degradation of lipids from cell membranes)

Please replace the heading beginning on page 26, line 1, with the following amended heading:

~~Example 3. Inhibition of proteases (this experiment is analogous to an experiment described in the European patent application no. 03292668.5)~~ **EXAMPLE 3. INHIBITION OF PROTEASES (THIS EXPERIMENT IS ANALOGOUS TO AN EXPERIMENT DESCRIBED IN THE EUROPEAN PATENT APPLICATION NO. 03292668.5)**

Please replace the paragraph beginning on page 26, line 21, with the following amended paragraph:

Elastase inhibition in ~~tube~~ vitro (in % of inhibition compared to control (=0%), average of 2 assays):

concentration of the tested product in % (w/v)	aqueous extract, batch A	aqueous extract, batch B	alcoholic extract, batch A	alcoholic extract, batch B
control	0	0	0	0
0.3	30	33	23	0

Please replace the paragraph beginning on page 26, line 28, with the following amended paragraph:

Castanea extracts have inhibited elastase activity in tube vitro, and could be used to fight against skin ageing and skin photo-ageing.

Please replace the heading beginning on page 27, line 1, with the following amended heading:

~~Example 4: Inhibition of melanin synthesis (this experiment is analogous to an experiment described in the European patent application no. 03292735.2)~~ **EXAMPLE 4: INHIBITION OF MELANIN SYNTHESIS (THIS EXPERIMENT IS ANALOGOUS TO AN EXPERIMENT DESCRIBED IN THE EUROPEAN PATENT APPLICATION NO. 03292735.2)**

Please replace the paragraph beginning on page 27, line 8, with the following amended paragraph:

**Protocol of efficacy test on B16 melanocytes:**

- Seeding of melanocytes in growth medium
- Incubation for 3 days at 37 °C, ~~CO-2~~ CO<sub>2</sub> = 5 % (atmosphere: 5 % carbon dioxide in air)
- Exchange of the growth medium by a medium with a range of concentration of ingredients to be tested
- Incubation for 3 days at 37 °C, ~~CO-2~~ CO<sub>2</sub> = 5 % (atmosphere: 5 % carbon dioxide in air)
- Recording of Cellular levels of protein by Bradford's method
- Recording of melanin by a spectrophotometric method (OD at 475 nm)

Please replace the heading on page 28, line 1, with the following amended heading:

**Claims WE CLAIM:**

Please replace the paragraph beginning on page 30, line 3, with the following amended paragraph:

The present invention is ~~concerned with~~ a composition comprising an extract of the leaves



**Preliminary Amendment of U.S. National Stage for International Application  
PCT/EP2005/001105 filed February 4, 2005**

of the ~~plant~~ Castanea sativa plant. It is furthermore ~~concerned with~~ directed to the use of ~~this~~ the extract for the manufacture of a cosmetic composition and with the use of this extract for the cosmetic treatment of the human body.